Assessing the Possibility of RUNX1, ETV6 and GATA2 Related Germline Predisposition in Myeloid Neoplasms in a Somatic Cancer Setting

Authors: Kaylee Barber, MS, CGC; Jianhua Zhao, PhD, DABMG; Jeffrey Bissonnette, MSc, CGC, CCGC; Rachel T. Klein MS, CGC

ONCOLOGY genpath an OPKO Health Company

Recently the World Health Organization (WHO) released an update to the classification of hematopoietic neoplasms with germline predisposition based on the presence of germline pathogenic variants in RUNX1, ETVS and (WHO, 2016). Individuals who are heterozygous for a germline RUNX1 variant are at a 35-44% risk of GATA2 myelodysplastic syndrome (MDS [614286]) or acute myeloid leukemia (AML [601626]) (Owen et al, 2008, Godley, 2014). Germline ETV6 variants are associated with Thrombocytopenia 5 [616216], an autosomal dominant thrombocytopenia. Clinical presentation for those with germline GATA2 variants is variable, however lifetime risk for MDS or AML is estimated to be 70%.

We performed a retrospective analysis of all RUNX1, ETV6 and GATA2 variants identified on a Next Generation Sequencing panel for a myeloid neoplasm. Our goal was to determine how the new classification released by WHO would affect our strategy in reporting these variants.

RUNX1, ETV6 and GATA2 variants were detected in 637 cases in our lab out of the total 7,092 myeloid cases analyzed over the last 17 months (637/7,092 = 9%). RUNX1 variants are most commonly detected among these three genes (499/637= 78%). The median age of all patients in this study was 71.

Of the cases where a diagnosis was known, RUNX1/GATA2/ETV6 variants were most commonly seen in AML. Of a total of 756 unique RUNX1, ETV6 or GATA2 variants reported, 67% were known disease associated variants while 33% were novel or unclear variants. Of the 507 disease associated variants, 155 were found to have an allele frequency suggestive of a possible germline variant (31%). Of the ten youngest patients (ages 6-26) identified with one of these variants, seven (70%) were detected at an allele frequency suggestive of a germline mutation (40-60% allele frequency).

Based on the relatively high proportion of cases with an allele frequency suggestive of a germline variant and the significance of a germline variant, we conclude that including a statement on the final report regarding the possibility of a germline mutation is reasonable in all cases where one of these variants is detected. Germline confirmation testing should be pursued when indicated. This will allow for appropriate management of those with germline predisposition to myeloid malignancies, and would also promote testing of family members that could be at risk. In addition, this study shows the value of having genetic counseling expertise within a somatic testing lab.

INTRODUCTION

- The purpose of our study was to see how often we are detecting RUNX1, ETV6 and GATA2 alterations in younger patients and at allele frequencies suggestive of a germline mutation. This study allowed us to determine how the new classification released by WHO would affect our strategy in reporting these variants that appear to be possibly present in the germline.

METHODS

- For our study we isolated the number of RUNX1, ETV6 and GATA2 alterations we have detected through our somatic next generation sequencing on myeloid neoplasms. We then isolated the RUNX1, ETV6, and GATA2 alterations that were present at an allele frequency that could be suggestive of a germline mutation (40-60% allele frequency). We also gathered clinical information on the top ten youngest patients with either a RUNX1, ETV6 or GATA2 alteration.

RESULTS

- RUNX1, ETV6 and GATA2 variants were detected in 637 cases in our lab out of the total 7,092 myeloid cases analyzed over the last 17 months (637/7,092 = 9%). RUNX1 variants are most commonly detected among these three genes (499/637= 78%).

Diagram 1, 2, and 3 show that the majority of RUNX1 variants were disease associated and the majority of ETV6 and GATA2 variants were unclear variants. Of a total of 756 unique RUNX1, ETV6 or GATA2 variants reported, 67% were known disease associated variants while 33% were novel or unclear variants.

Diagram 1: Proportion of RUNX1 unclear variants versus disease associated alterations.

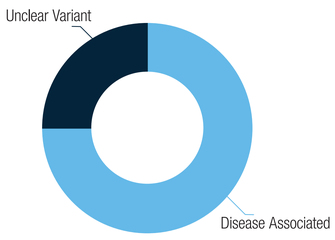


Diagram 2: Proportion of ETV6 unclear variants versus disease associated alterations.

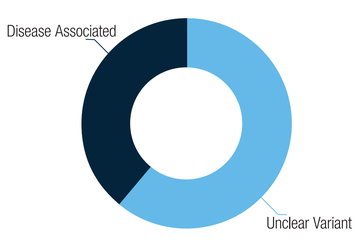


Diagram 3: Proportion of GATA2 unclear variants versus disease associated alterations.

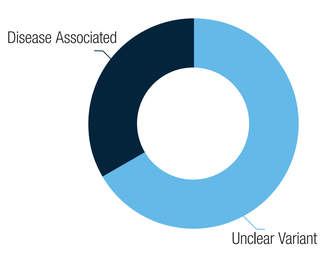


Table 1: Tumor types seen in cases with RUNX1 alterations, separated by age.

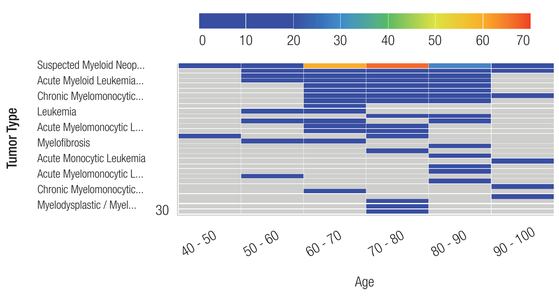


Table 2: Tumor types seen in cases with ETV6 alterations, separated by age.

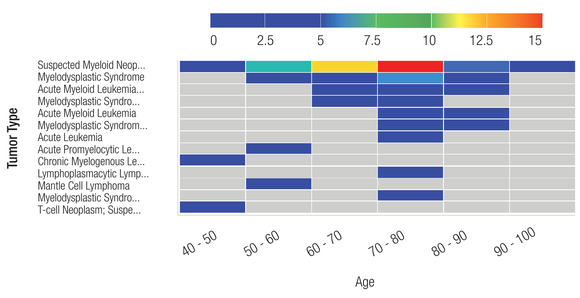
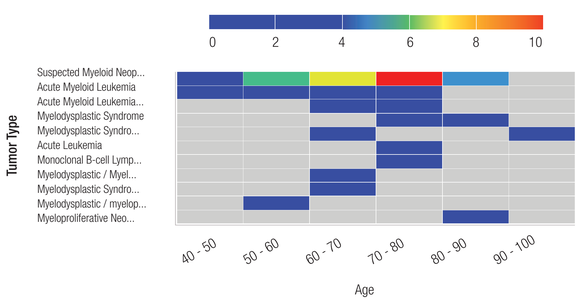


Table 3: Tumor types seen in cases with GATA2 alterations, separated by age.



Tables 1, 2, and 3 show that the majority of individuals with RUNX1, ETV6 and GATA2 alterations were between 60-90 years of age. These tables also show that the most common category of disease was a suspected myeloid neoplasm. **Of the cases where a diagnosis was known, these alterations were most commonly seen in myelodysplastic syndrome and acute myeloid leukemia (Table 1, 2, and 3).**

Of the 507 disease associated variants, 155 were found to have an allele frequency suggestive of a possible germline variant (31%). Of the RUNX1, ETV6 and GATA2 disease associated variants seen at an allele frequency suggestive of a germline alteration the majority were seen in patients between the age of 60 to 90. However, there were also several cases in diagrams 4, 5, and 6 that were between the age of 20-50.

Diagram 4: Age categories of patients with RUNX1 disease associated variants (98 cases total) with an allele frequency suggestive of a germline mutation (40-60%).

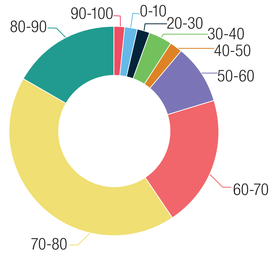


Diagram 5: Age categories of patients with ETV6 disease associated variants (11 cases total) with an allele frequency suggestive of a germline mutation (40-60%).

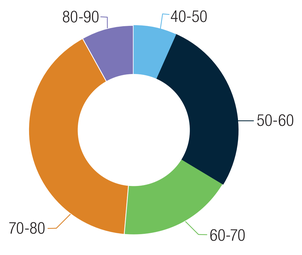


Diagram 6: Age categories of patients with GATA2 disease associated variants (10 cases total) with an allele frequency suggestive of a germline mutation (40-60%).

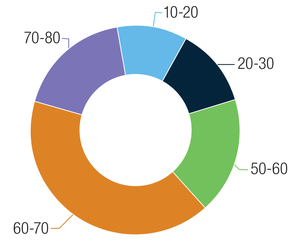


Table 3 shows that the youngest patient we saw with a disease associated variant was 12 years old (GATA2 variant) while the oldest patient was 96 years old (RUNX1 variant), with a median age of 71. **Of the ten youngest patients (ages 12-29) identified with one of these variants, five (50%) were detected at an allele frequency suggestive of a germline mutation (40-60% allele frequency) (Table 3).**

Table 4: Clinical information of the top ten youngest patients identified to have a RUNX1, ETV6, or GATA2 disease associated variant between 40-60% allele frequency.

Alteration	Allele Frequency (%)	Age	Gender	Test ordered	Other gene alterations detected	Tumor type	Other Clinical Information
GATA2 p.R398W	45.58	12	Male	Myeloid Disorder Sequencing Panel	-	Suspected myeloid neoplasm	GATA2 insufficiency reported
RUNX1 p.Q208Rfs*4	51.86	23	Male	Myeloid Disorder Sequencing Panel	KIT, PTPN11, WT1	Leukemia	None
RUNX1 p.T45Hfs*93	86	25	Male	Myeloid Disorder Sequencing Panel	ASXL1, SRSF2	Suspected myeloid neoplasm	Myelodysplastic Syndrome
GATA2 p.R337*	48.45	26	Female	Myeloid Disorder Sequencing Panel	-	Suspected myeloid neoplasm	Myelodysplastic syndrome
RUNX1 p.A251Lfs*12	12.8	26	Male	AML sequencing panel	ASXL1, EZH2, PTEN	T-cell acute lymphoid leukemia	peripheral T-cell lymphoma
RUNX1 p.E422Gfs*174	35.76	28	Female	Myeloid Disorder Sequencing Panel	DNMT3A, FLT3	Acute myeloid leukemia	-
RUNX1 p.?	35.39	28	Male	AML sequencing panel	RUNX1 p.? at 41.78% AF in another sample from this patient	Acute myeloid leukemia	Karyotype (45,XY,-7[20])
RUNX1 p.K110Q	76.04	28	Female	Myeloid Disorder Sequencing Panel	BCOR, BCORL1, FLT3	Acute myeloid leukemia	-
RUNX1 p.R204Q	82.44	29	Male	AML sequencing panel	EZH2, WT1	Acute myeloid leukemia	Positive for MLL (11q23) gene rearrangement

Discussion

The major finding of our study was that suspected germline RUNX1, ETV6 and RUNX1 variants have been detected in young patients (ages 20-50) and in patients with a myeloid neoplasm, usually either acute myeloid leukemia or myelodysplastic syndrome. However, it is important to note that older age and lack of family history of disease or clinical history of disease does not necessarily rule out a germline predisposition. Additionally, due to variations in the tumor burden as well as the presence of other genetic alterations and preferential detection of alleles, the allele frequency of ~50% is not definitive enough to confirm a germline mutation. Although germline mutations were not confirmed with skin biopsies, these findings still identify a need to inform doctors and patients about the possibility of a germline predisposition.

The WHO guidelines suggest genetic counseling for any patient with acute leukemia or MDS with a first-degree or second-degree relative with AML, acute lymphocytic leukemia, or MDS. They also suggest keeping in mind other non-hematological characteristics that may be suggestive of germline predispositions to myeloid malignancies (WHO, 2016; Bannon and DiNardo, 2016).

Currently, for possible germline RUNX1, ETV6 and GATA2 variants our interpretation statement reads "Germline mutations in RUNX1/ETV6/GATA2 can predispose to a myeloid malignancy and genetic testing on constitutional tissue can be performed in order to exclude somatic mutations (NCCN Guidelines, Myelodysplastic Syndromes, Version 2.2017).

References

Bannon SA, DiNardo CD. Hereditary Predispositions to Myelodysplastic Syndrome. Int J Mol Sci. 2016 May 30;17(6). pii: E838. doi: 10.3390/ijms17060838. Review.PubMed PMID: 27248996; PubMed Central PMCID: PMC4926372.

Czuchlewski DR, Peterson LC. Myeloid Neoplasms with Germline Predisposition: A New Provisional Entity Within the World Health Organization Classification. Surg Pathol Clin. 2016 Mar;9(1):165-76. doi: 10.1016/j.path.2015.09.010. Review. PubMed PMID: 26940275.

Owen CJ, Toze CL, Koochin A, Forrest DL, Smith CA, Stevens JM, Jackson SC, Poon MC, Sinclair GD, Leber B, Johnson PR, Macheta A, Yin JA, Barnett MJ, Lister TA, Fitzgibbon J. Five new pedigrees with inherited RUNX1 mutations causing familial platelet disorder with propensity to myeloid malignancy. Blood. 2008 Dec 1;112(12):4639-45. doi: 10.1182/blood-2008-05-156745. Epub 2008 Aug 21. PubMed PMID: 18723428.

Godley LA. Inherited predisposition to acute myeloid leukemia. Semin Hematol. 2014 Oct;51(4):306-21. doi:10.1053/j.seminhematol.2014.08.001. Epub 2014 Aug 12. PubMed PMID: 25311743.

West AH, Godley LA, Churpek JE. Familial myelodysplastic syndrome/acute

leukemia syndromes: a review and utility for translational investigations. Ann N Y Acad Sci. 2014 Mar;1310:111-8. doi: 10.1111/nyas.12346. Epub 2014 Jan 27. Review. PubMed PMID: 24467820; PubMed Central PMCID: PMC3961519.

